

Porcine Rota virus	+		
Porcine Vesicular Exanthema virus	+	+	
Rabies virus	+		+
Swine Pox virus	+	+	
Swine Vesicular Disease virus	+	+	
Vesicular Stomatitis virus	+		+

Table 4. Extraneous agents relevant for equines and of material of equine origin.

AGENT	SPF HERD	CELLS AND SEEDS	
		GENERAL TESTS	SPECIFIC TESTS
African Horse Sickness virus	+		+
Borna Disease virus	+		
Equine Arteritis virus	+	+	
Equine encephalomyelitis virus (Eastern, Western, Venezuelan)	+		+
Equine Herpes virus types 1, 2 , 3 and 4	+	+	
Equine Infectious Anaemia virus	+		+
Equine Influenza virus	+		+
Japanese B. Encephalitis virus	+		+
Rabies virus	+		+
Vesicular Stomatitis virus	+		+

Table 5 Extraneous agents relevant for felines and of material of feline origin.

AGENT	SPF HERD	CELLS AND SEEDS	
		GENERAL TESTS	SPECIFIC TESTS
Aujeszky's Disease virus	+	+	
Cowpox virus	+	+	
Feline Calici virus	+	+	
Feline Herpes virus type 1	+	+	
Feline Immunodeficiency virus	+		+
Feline Leukaemia virus / Feline Sarcoma virus	+		+
Feline Infectious Peritonitis virus / Feline Enteric Corona virus	+		
Feline Panleucopenia virus	+	+	
Feline Syncytia Forming virus	+		+
Rabies virus	+		+

Table 6 Extraneous agents relevant for canines and of material of canine origin.

AGENT	SPF HERD	CELLS AND SEEDS	
		GENERAL TESTS	SPECIFIC TESTS
Aujeszky's Disease virus	+	+	
Canine Adeno virus type 1 and 2	+	+	
Canine Corona virus	+	+	
Canine Distemper virus	+	+	
Canine Herpes virus	+	+	+
Canine Parvo virus	+	+	+
Parainfluenza 2 virus (SV-5)	+	+	
Rabies virus	+		+

Table 7 Extraneous agents relevant for rabbits and of material of rabbit origin.

AGENT	SPF HERD	CELLS AND SEEDS	
		GENERAL TESTS	SPECIFIC TESTS
Arena virus (Lymphocytic Choriomeningitis virus)	+		+
Aujeszky's Disease virus	+	+	
Encephalomyocarditis virus	+	+	
Myxoma virus (Shope Fibroma virus)	+		+
Rabbit Haemorrhagic Disease virus	+	-	+
Rabies virus	+		+

ANNEXE 2.

Precise description of the specific diagnostic test methods used in the examination for extraneous agents.

To be developed

ANNEX 3

PROPOSAL FOR HARMONISED TESTING REQUIREMENTS.

The attached tabulations list the test requirements specified by the European, United States (and Japanese) authorities and propose in each case a test which would include the requirements of all (three) authorities.

The tabulations list, in turn, the tests specified by the (three) authorities for the testing of master and working cell seeds, and master virus seeds, together with agents for which specific tests are required according to the species of origin.

In each case a proposed harmonised test is indicated which embraces the requirements of all (three) authorities and which can be recognised by each authority as complying with its own specification.

For agents for which specific tests are required, the proposed harmonised test column indicates that either an appropriate specific test must be performed or that an assessment of the epidemiology of the agent to demonstrate that the test material carries no risk, is acceptable.

Where a specific test is conducted, the most sensitive available procedure should be chosen. In the majority of cases this can be satisfied with an immunochemical test .

References:

European Pharmacopoeia - Monograph 62 - Vaccines for Veterinary Use (1995)

CVMP - General requirements for the production and control of live mammalian bacterial and viral vaccines for veterinary use (1993)
CVMP - General requirements for the production and control of inactivated mammalian bacterial and viral vaccines for veterinary use (1993)
CVMP - Note for Guidance - Table of extraneous agents to be tested for in relation to the general and species specific guidelines on the
Production and control of mammalian veterinary vaccines (1993)
Code of Federal Regulations (1996)
9CFR 113.55 Detection of extraneous agents in Master Seed Virus
9CFR 113.46 Detection of cytopathogenic and/or haemadsorbing agents
9CFR 113.47 Detection of extraneous viruses by the fluorescent antibody technique
9CFR 113.51 Requirements for primary cells used for production of biologics
9CFR 113.52 Requirements for cell lines used for production of biologics
Japanese requirements for testing of vaccines for extraneous agents - Intervet K.K. (personal communication)

COMPARISON OF THE REQUIREMENTS FOR EXTRANEIOUS AGENT TESTING OF VETERINARY VACCINE CELL AND VIRUS SEED MATERIALS FOR EUROPE, THE USA AND JAPAN, AND PROPOSALS FOR HARMONISATION

1a Master and working cell seeds

Direct testing

PROCEDURE	EP/CVMP**	9CFR	JAPAN	PROPOSED HARMONISED TEST
Size of monolayer	70 cm ²	75 cm ²		75 cm ²
Subculture frequency	every 7 days	at least twice		Subculture at least twice. (every 7 days)
Examination for cpe	regularly	regularly		regularly
Duration of test	28 days	21 days		28 days
cpe	2 x 6 cm ²	1 x 6 cm ²		2 x 6 cm ²
Haemadsorption	at least 70 cm ²	at least 6 cm ² at least 7 days from last subculture		at least 70 cm ² at least 7 days from last subculture
Red blood cell species	suitable	mixed or separate chicken and guinea pig		as appropriate but including chicken and guinea pig
Haemadsorption incubation	not specified	4 °C for 30 min. then 20-25 °C for 30 min.		4 °C for 30 min. then 20-25 °C for 30 min.
Immunofluorescence procedure	not specified	monolayer at least 7 days from last subculture; total surface area at least 6 cm ²		monolayer at least 7 days from last subculture; total surface area at least 6 cm ²
Further preparation of material	at least 140 cm ² , freeze/thaw x 3, centrifuge	at least 75 cm ² , freeze/thaw x 3, centrifuge <2000g for <15 min.		at least 140 cm ² , freeze/thaw x 3, centrifuge <2000g for <15 min.
Specific agents (immunofluorescence)	see (3) below	see (3) below		see (3) below
Other	Monkey cells tested for C, D			Monkey cells tested for C, D

	type particles (retroviruses)			type particles (retroviruses)
--	-------------------------------	--	--	-------------------------------

1b Master and working cell seeds

Indirect testing

PROCEDURE	EP/CVMP**	9CFR	JAPAN	PROPOSED HARMONISED TEST
Cell lines	Primary cells of source species - sensitive to target species pathogens. cells sensitive to pestiviruses (See also 2.2.1)	Embryo, neonatal or primary of source species; Vero cells: embryo, neonatal or cell line of bovine origin		primary cells of source species; cells sensitive to virus pathogenic for target species; bovine cells sensitive to pestivirus.
Inoculum*	unspecified	1.0 ml onto >75 cm ²		1.0 ml on flask > 75 cm ²
Incubation time	7 days then freeze/thaw; new cultures 7 days	14 days with one subculture		14 days with one passage at FT
Cpe	2 x 6 cm ²	1 x 6 cm ²		2 x 6 cm ²
Haemadsorption	at least 70 cm ²	at least 6 cm ² at least 7 days from the last subculture		at least 70 cm ² (total) at the end of of the last passage
Specific agents (immunofluorescence)	see (3) below	see (3) below		
Other It should be specified whether this are general or specific tests and if the latter, for which agents. (to be decided)	Monkey cells tested for C and D type particles (retroviruses)		Primary guinea pig cells: inoculate >30 tubes with 0.1 ml sample, incubate 10 days at 37 °C; observe for CPE and HAd with guinea pig, goose or chick (<7 dayold) rbc. Suckling mice, <3 days old. Inoculate 0.02 ml intracerebrally, observe 5 days, remove brains, homogenise and inoculate supernate	Monkey cells tested for C and D type particles (retroviruses) Primary guinea pig cells: inoculate >30 tubes with 0.1 ml sample, incubate 10 days at 37 °C; observe for CPE and HAd with guinea pig, goose or chick (<7 dayold) rbc. Suckling mice, <3 days old. Inoculate 0.02 ml intracerebrally, observe 5 days, remove brains, homogenise and inoculate supernate

			intracerebrally into 10 mice; observe for 10 days.	intracerebrally into 10 mice; observe for 10 days.
--	--	--	---	---

* The method of preparing the inoculum shall be specified e.g. freezing/thawing etc. (*to be decided*)

2 Master virus seeds

PROCEDURE	EP/CVMP**	9CFR	JAPAN	PROPOSED HARMONISED TEST
Test volume *	10 vaccine doses/ml on to 70 cm ² monolayer	1.0 ml per 75 cm ² monolayer	8 x 20 cm ² monolayer; 0.2 ml/culture	10 doses of vaccine per ml if possible on to 8 x 20 cm ² monolayers
Duration of test	Observe daily for one week; freeze/thaw x 3, re-inoculate; repeat twice	Maintain for 14 days with >1 subculture Vero cells	Sub-culture every 3-5 days to third generation	total duration: 28 days; with three passages. Passages are performed on days 7, 14 and 21 regular observations.
Cell lines	Primary cells of source species; cells sensitive to target species pathogens; cells sensitive to pestiviruses	Embryo, neonatal or cell line of target species	Primary or established cell line sensitive to target species pathogens	Primary cells of source species (or VERO cells ?); cells sensitive to viruses pathogenic for target species; bovine cells sensitive to pestivirus.
Cpe	2 x 6 cm ²	1 x 6 cm ²	more than 4 dishes	2 x 6 cm ²
Haemadsorption	at least 70 cm ²	at least 6 cm ² at least 7 days from last subculture	more than 4 dishes incubated 7 days post inoculation	at least 70 cm ² (total) at the end of the last passage
Specific agents (immunofluorescence)	see (3) below	see (3) below	see (3) below	see (3) below
Other			Primary guinea pig cells: inoculate >30 tubes with 0.1 ml sample, incubate 10 days at 37 °C; observe for CPE and HAd with guinea pig, goose or chick (<7 dayold) rbc. Suckling mice, <3 days old. Inoculate 0.02 ml intracerebrally, observe 5 days, remove brains, homogenise and inoculate supernate intracerebrally into 10 mice; observe for 10 days.	Primary guinea pig cells: inoculate >30 tubes with 0.1 ml sample, incubate 10 days at 37 °C; observe for CPE and HAd with guinea pig, goose or chick (<7 dayold) rbc. Suckling mice, <3 days old. Inoculate 0.02 ml intracerebrally, observe 5 days, remove brains, homogenise and inoculate supernate intracerebrally into 10 mice; observe for 10 days.

- The proposed test volumes may be problematic. The number of passages included in the proposed harmonised test should provide some margin of safety. This matter has to be discussed.
- The rationale of the suckling mice/mice inoculation shall be investigated in order to reduce use of experimental animals. (Animal Welfare)

3

[illegible]

*Chlamydia ovis*³

Specific i.e. histopathological staining

3 (cont)

PROCEDURE	EP/CVMP**	9CFR	JAPAN	PROPOSED HARMONISED TEST
canine origin	Rabies virus <i>Brucella canis</i>	canine coronavirus canine distemper virus canine parvovirus	Dog kidney primary or cell line >40 cm ² , 2.0 ml inoculum. Incubate 5 days, subculture, incubate 10 days, cpe and/or HAd with guinea pig rbc	Specific tests (see below) Specific Specific Specific Specific Specific
equine origin	African horse sickness virus equine encephalomyelitis virus equine infectious anaemia virus equine influenza virus Japanese B encephalomyelitis virus rabies virus vesicular stomatitis virus	equine herpesvirus equine viral arteritis		Epidemiology Epidemiology Specific Specific Epidemiology Specific Epidemiology Specific Specific
feline origin	feline immunodeficiency virus feline leukaemia virus feline syncytia forming virus rabies virus <i>Chlamydia psittaci</i>	feline infectious peritonitis virus feline panleucopaenia virus	Cat kidney primary or cell line >40 cm ² , 2.0 ml inoculum. Incubate 5 days, subculture, incubate 10 days, cpe and/or HA with swine rbc after 18 hr at 4°C	Specific (see below) Specific Specific Specific Specific i.e.histopathological staining Specific Specific Specific i.e.histopathological staining

3 (cont)

PROCEDURE	EP/CVMP**	9CFR	JAPAN	PROPOSED HARMONISED TEST
porcine origin	<p>African swine fever virus</p> <p>bovine viral diarrhoea virus</p> <p>classical swine fever virus</p> <p>foot and mouth disease viruses</p> <p>porcine cytomegaloviruses</p> <p>porcine influenza virus</p> <p>porcine parvovirus</p> <p>porcine respiratory and reproductive syndrome virus</p> <p>rabies virus</p> <p>vesicular stomatitis virus</p> <p><i>Brucella suis</i></p> <p><i>Mycoplasma hyorhinis</i></p> <p><i>Mycoplasma hyopneumoniae</i></p>	<p>porcine parvovirus</p> <p>porcine adenovirus</p> <p>transmissible gastro-enteritis virus</p> <p>porcine haemagglutinating encephalitis virus</p>	<p>Swine kidney primary or cell line; inoculate 4 ml sample, >20 cm²/ml. Incubate 5 days at 37°C, observe for CPE, subculture, incubate 7 days at 37 °C, observe for CPE. Divide cultures into three groups, HAd with guinea pig, goose and chicken (7 day old) rbc</p> <p>Conduct FA test for hog cholera (swine fever) virus</p>	<p>Specific tests (see below)</p> <p>Epidemiology</p> <p>Specific</p> <p>Specific</p> <p>Epidemiology</p> <p>Specific</p> <p>Specific</p> <p>Specific</p> <p>Specific</p> <p>Specific</p> <p>Epidemiology</p> <p>Epidemiology</p> <p>Specific</p> <p>Specific</p> <p>Specific</p> <p>Specific</p>
Cell seeds indirect test	as above	as above		
Virus seeds	as above	as above		

* fixed positive rabies controls available from NVSL

** specific tests may be omitted if adequately justified